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Sequence Homology Between the Structural Proteins of Kilham Rat Virus

DALE D. BROWN* AND LOIS A. SALZMAN

Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

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The capsid proteins of the autonomous parvovirus Kilham rat virus were purified and analyzed for peptide composition. Partial proteolysis mapping and two-dimensional thin-layer chromatography of tryptic peptide digests revealed extensive amino acid sequence homology between the two major Kilham rat virus capsid proteins.

Parvoviruses are among the smallest eucaryotic viruses. They have been isolated from a wide variety of hosts. The virions are composed of one linear single-stranded DNA genome that is encapsidated by protein in an icosahedral nonenveloped particle (12). Vertebrate parvoviruses are divided into two subgroups on the basis of their ability to replicate autonomously in the infected cell. The adeno-associated virus subgroup is defective and requires coinfection with adenovirus for its replication. The autonomous virus subgroup does not require helper viruses for replication, although they require the host cell to be in the late S or G2 phase for infection to occur (17). The structural proteins of the autonomous parvoviruses minute virus of mice (MVM), H1, and Kilham rat virus (KRV) have been analyzed (3, 4, 14). Generally, two or three polypeptides have been found. Recently, nonstructural virus-specific proteins have been reported in cells infected by some of the autonomous parvoviruses (1, 7, 8, 10).

Because of the small size of the parvovirus DNA (ca. 4,000 to 5,000 nucleotides in length), its coding capacity is limited to a few proteins. If the reported molecular weights of the viral capsid and structural proteins are correct and the proteins are all coded for by the viral genome, more than 100% of the theoretical coding capacity of the viral genome would be required. Two ways the virus could overcome this problem would be to have overlapping genes or to produce several proteins from the same set of codons. The parvoviruses MVM and adeno-associated virus (6, 16) have been shown to have peptide sequence homology in their major capsid proteins. They are believed to produce these proteins from identical codon sequences (6, 16). We have investigated these possibilities by determining whether the viral proteins contain common amino acid sequences as determined by peptide digests of purified proteins.

To study the viral proteins in detail, large-scale growth and purification of the virus was necessary. KRV strain 308 was grown in a rat nephroma cell line as previously described (13). At 72 h postinfection, the supernatant was harvested, and virions were pelleted and twice centrifuged in isopycnic CsCl gradients. After the initial CsCl gradient, four viral bands were visualized. Similar patterns of viral bands in CsCl gradients have been reported with other parvoviruses (11). The low-density bands represent particles containing no DNA or aberrant lengths of DNA molecules, whereas high-density bands contain full particles.

Analysis of structural proteins from the empty (no DNA) and full viral particles (complete viral genome) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis is shown in Fig. 1. The empty (lane 2) and full (lane 3) particles both contain the A (89,000 dalton [89K]) and B (68K) proteins.

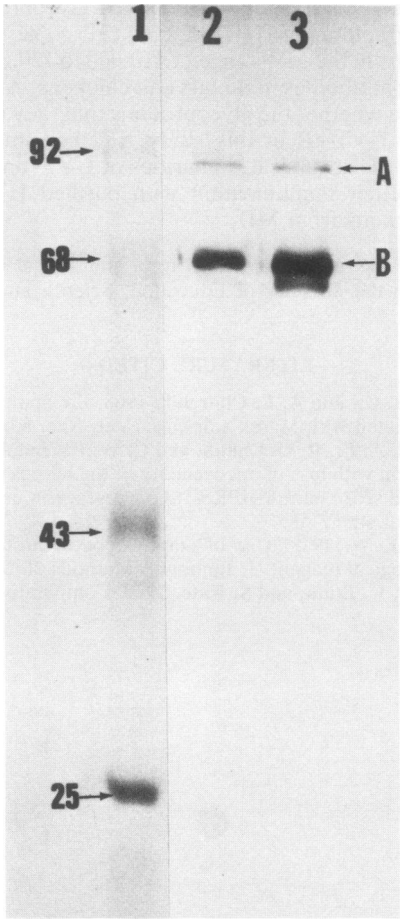

FIG. 1. Autoradiograph of 15% sodium dodecyl sulfate-polyacrylamide gel analysis of KRV virion proteins. Purified virus was disrupted in sodium dodecyl sulfate and β-mercaptoethanol as described previously (14). Samples from the empty particles (lane b) and full particles (lane c) were electrophoresed through a 15% sodium dodecyl sulfate-polyacrylamide separating gel by the buffer system of Laemmli (5). The gel was dried and exposed to Kodak XAR-2 film at −70°C. Lane a contains protein markers of molecular weights of 200K, 92K, 68K, 43K, 25K, and 18K (Bethesda Research Laboratories).

* Corresponding author.

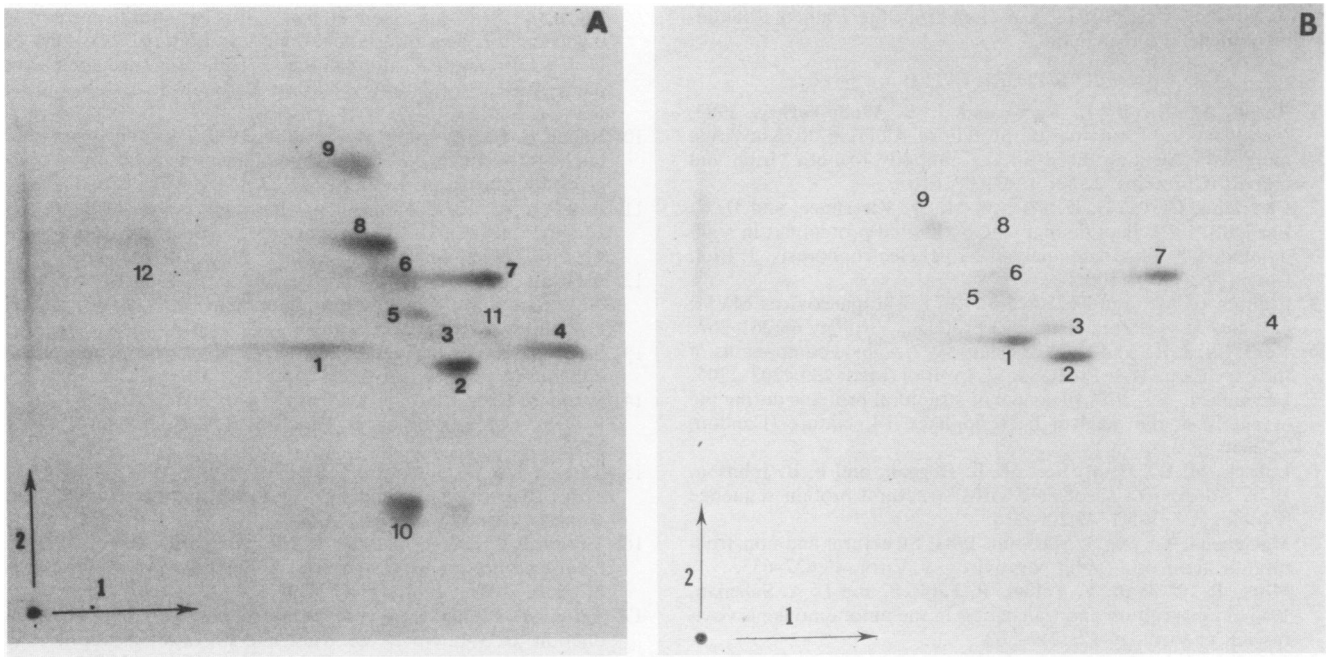

FIG. 2. Two-dimensional peptide maps of [^{35}S]methionine-labeled KRV proteins. Virions were disrupted as described above and electrophoresed on a 15% sodium dodecyl sulfate-polyacrylamide gel. Proteins were eluted as described by Bloom et al. (1). The gels were dried, marked with dots of ^{14}C ink, and autoradiographed at −70°C. The protein bands were located and excised. The gel pieces were cut into small pieces and incubated overnight at 37°C in several volumes of 0.05 M NH_4HCO_3 (pH 8)–1% β-mercaptoethanol–0.1% sodium dodecyl sulfate–100 μg of bovine serum albumin. The eluate was centrifuged, filtered, precipitated twice with trichloroacetic acid, washed with ethanol and ether (70:30), and finally washed with ether. Samples were then oxidized with performic acid for 2 h at 0°C. After lyophilization, proteins were digested with 10 μg of tolysulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Diagnostics) at 37°C for 1 h. An additional 10 μg of this enzyme was added, and the digestion was carried out for another 3 h. The tryptic digests were lyophilized, suspended in 20 μl of 0.1 M acetic acid, spotted onto thin-layer plates, and electrophoresed for 45 min at 1,000 V. Before electrophoresis, thin-layer plates were sprayed with electrophoresis buffer (formic acid-acetic acid-distilled water) (25:87:888 ml). After electrophoresis, plates were dried and chromatographed at a 90° angle to the direction of electrophoresis in butanol-pyridine-acetic acid-distilled water (75:50:15:60 ml) for 5 h. The dried plates were fluorographed with En^{3}Hance (New England Nuclear Corp.) and exposed at −70°C to Kodak XAR-2 film.

These proteins have been shown previously to be KRV specific. They have been synthesized in vitro in our laboratory with RNA from KRV-infected cells and were immunoprecipitated from KRV-infected cell extracts (8). The B protein is the major capsid protein. An additional protein (C) is seen on the autoradiograph very close to the B protein. The parvoviruses MVM and H1 are also reported to contain a protein that has a molecular weight of about 95% of that of the major protein. This protein, however, has been suggested to result from the cleavage of the B protein (3, 15). Subsequently, MVM proteins were shown to be structurally related (16). The amount of C protein seems to depend on growth conditions and perhaps manipulation of the virions during purification. Furthermore, trypsin has been shown to cleave the B protein of KRV, MVM, and H3 to C in vitro (9).

As mentioned above, proteins of various sizes could be obtained from the same DNA nucleotide sequences if the sequences contained more than one promoter site or are processed after translation of one protein. These proteins would share most of their amino acid sequences which could be demonstrated by proteolysis and chromatography. To determine whether the proteins were coded for by the same DNA sequences with the same reading frame, we analyzed the A and B proteins by the partial proteolysis peptide mapping procedure of Cleveland et al. (2). The proteins were digested with either *Staphylococcus aureus* V8 protease or chymotrypsin. Under these limited digestion conditions, polyacrylamide gel electrophoresis of the A and B capsid polypetides revealed bands that electrophoresed with the same mobility (data not shown). These data indicate that protein A and B are probably coded for by common nucleotide sequences. However, this technique only represents limited proteolysis, and the peptides are only compared on the basis of mobility in the polyacrylamide gel. A much more accurate way is by peptide fingerprinting. This technique is more definitive because it separates peptides on the basis of charge and solubility. Virions labeled with [^{35}S]methionine were purified, ruptured, and electrophoresed on polyacrylamide gels. The proteins were eluted from the gels, precipitated, oxidized, and trypsinized as described in the legend to Fig. 2. The tryptic peptide maps of the A and B protein are shown in Fig. 2. The map of the A and B proteins contain 12 and 9 tryptic peptides, respectively. All of the tryptic peptides of B protein are contained within A protein. Thus, KRV appears to make very efficient use of its DNA. It will be interesting to determine whether the proteins share common amino-terminal or carboxy-terminal ends. Recently, the structural proteins and a nonstructural protein of H1 (10) were mapped. The structural proteins were coded from the right half of the genome, and the nonstructural protein was coded from the left half. Studies are currently in progress to map KRV structural and nonstructural proteins to the viral genome. Further investigations of the genomic organization of KRV via analysis of virus-specific proteins may enhance the understanding of eucaryotic gene expression.

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